SafePharm Laboratories

Testing for a Safer Future

Expert Panel Meeting to Assess the Current Validation Status of In Vitro Testing Methods For Identifying Ocular Corrosives and Severe Irritants:
The Isolated Rabbit Eye (IRE)Test Method

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Scope of the Presentation

- Test Facility
- Eye Irritancy Testing Strategy
- Scientific basis for the IRE
- Apparatus
- Source of Tissues
- Pre-test Procedures
- Test substance application
- Observations
- Prediction Model
- Conclusion

SafePharm Laboratories Limited

- UK based CRO
- Established 30 Years
- Derby/Nottingham
- GLP Accredited
- www.safepharm.co.uk

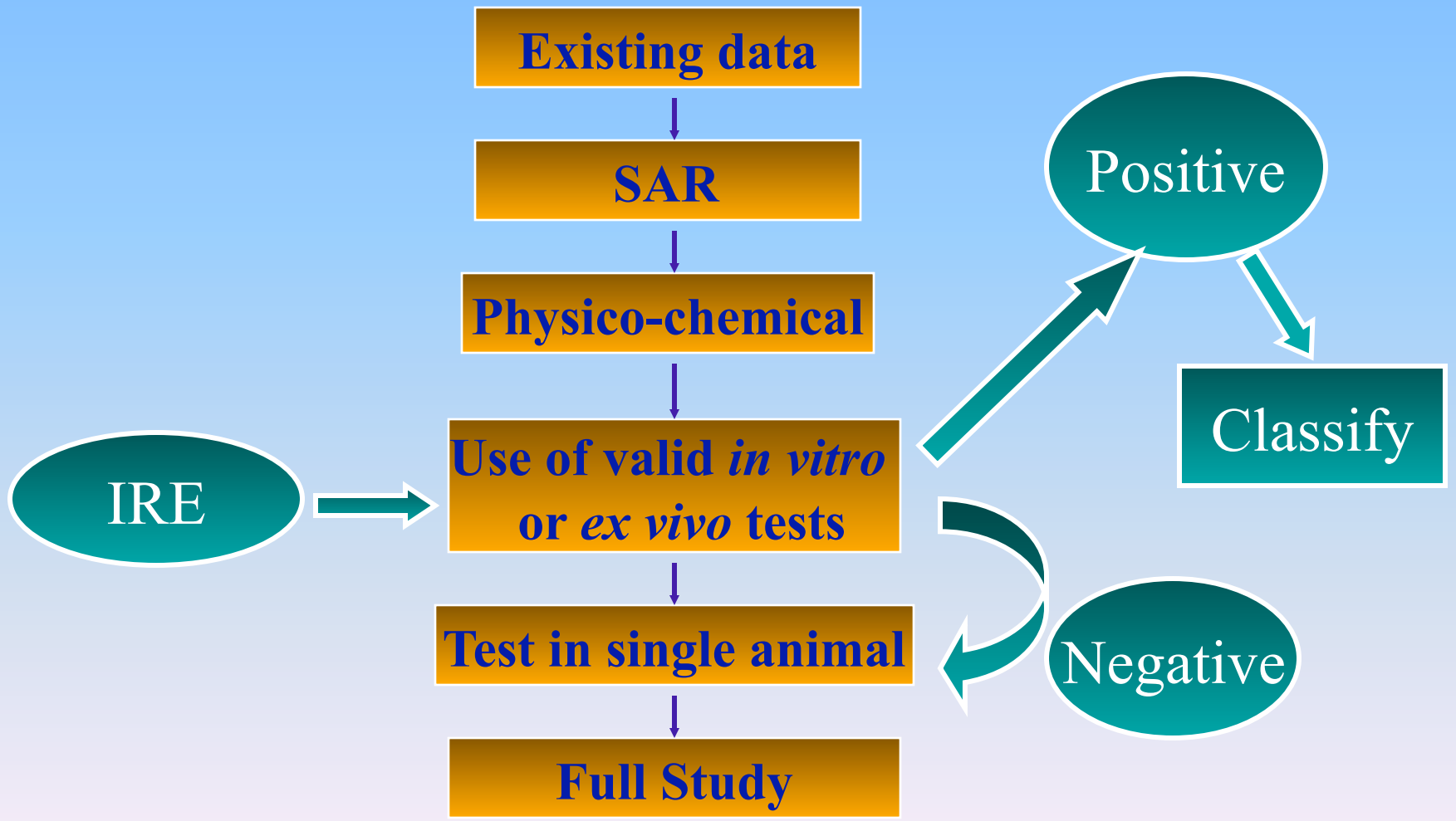
SafePharm Laboratories

Testing for a Safer Future

Eye Irritation Testing

- 300 - 400 eye irritation studies per year
- Wide range of test substances
- To satisfy regulatory requirements worldwide
- Occupational safety assessment
- In accordance with official test guidelines (e.g. OECD 405, 2002)
- Tiered Testing Strategy

Sequential Testing Strategy (OECD, 2002)

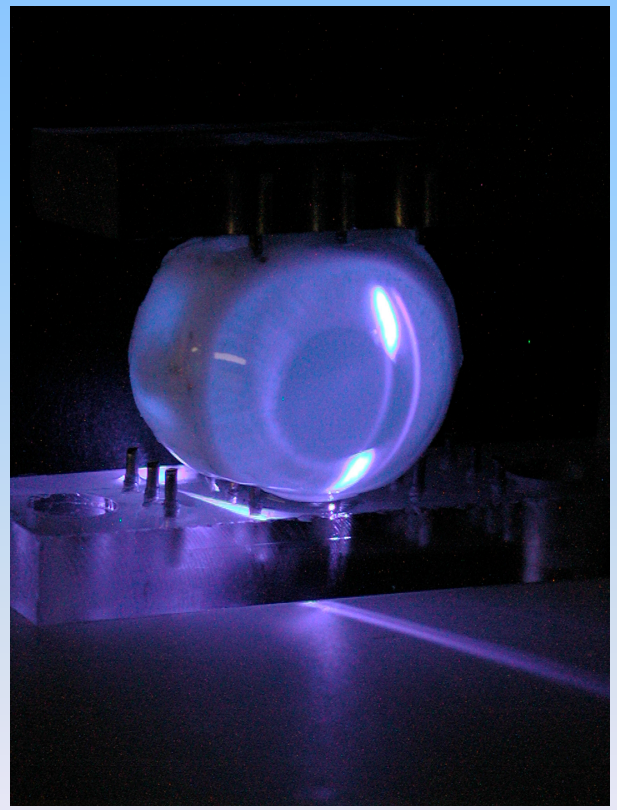


Isolated Rabbit Eye Test (IRE)

- Used ‘in-house’ since 1999
- Screening test for severe eye irritants & corrosives
- ‘Pre-validated’ in-house (14 chemicals)
- Validated on behalf of GSK for worker safety assessment

(30 chemicals)

- Has minimised exposure of animals to severe irritants



Scientific Basis for the IRE

Severely irritant materials have the potential to:

- damage the cells and structure of the cornea
- cause swelling of the cornea
- cause corneal opacity (cloudiness)

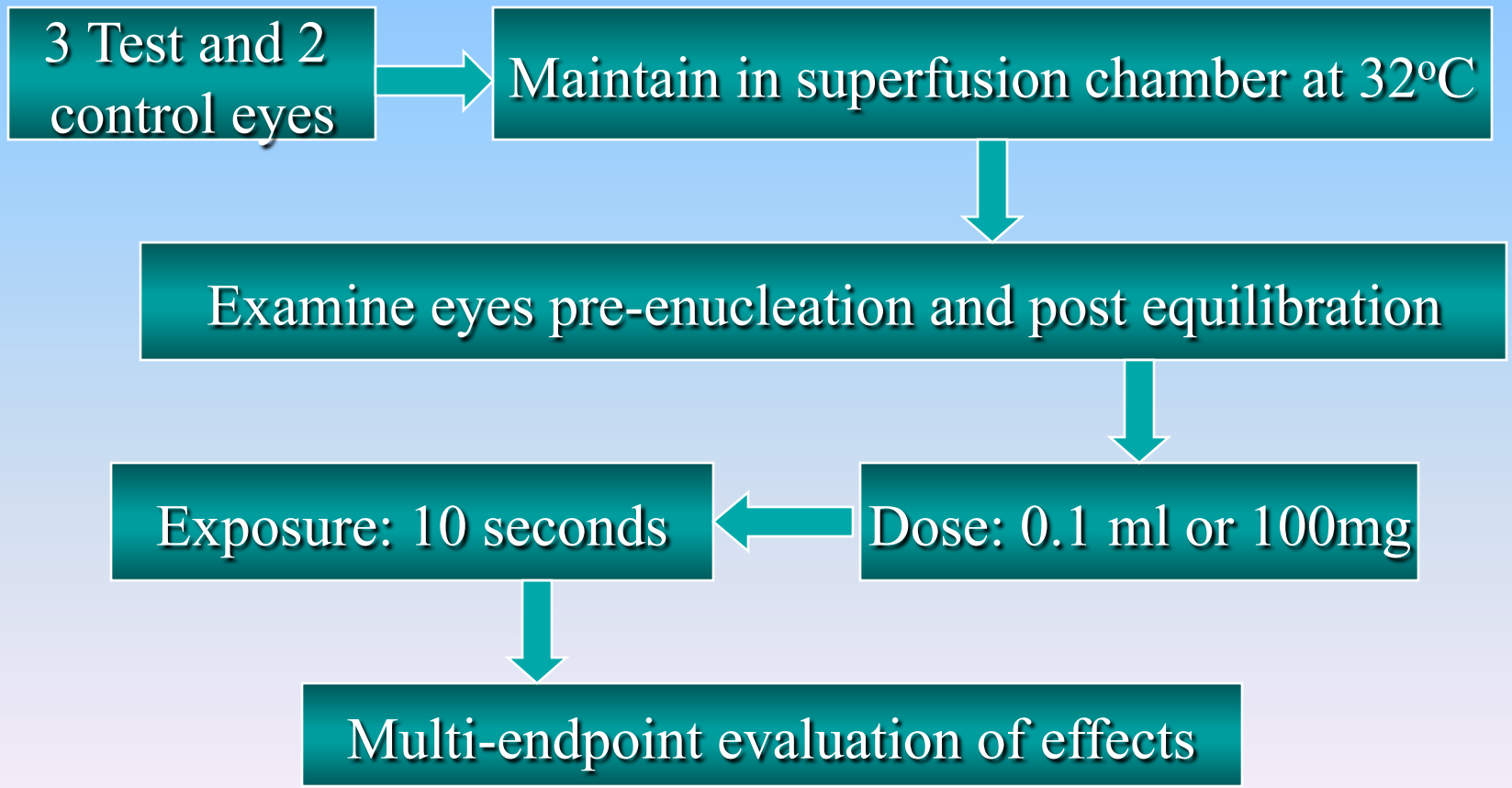
A whole cornea model is therefore relevant

The IRE involves use of rabbit eyes without causing pain or distress

Why IRE?

- Utilises target tissue (intact cornea)
- Corneal damage is ‘weighted’in the Draize Test
- Easily accessible source of fresh tissues
- Donor animals previously used for other purposes
- No major health and safety concerns
- Techniques transferable from *in vivo* testing
- Quantifiable endpoint (corneal thickness) supplements qualitative assessments
- Rapid (1 working day)

IRE Outline of Procedures



Multi-Endpoint Evaluation

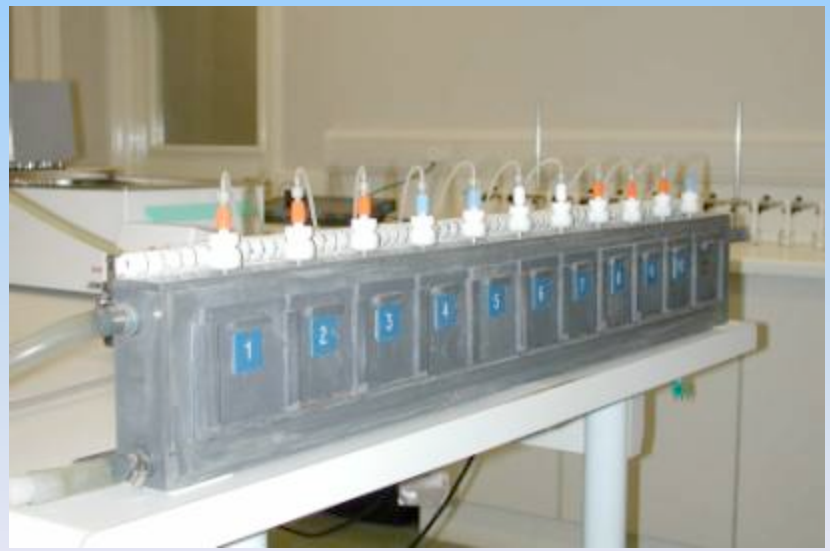
- Macroscopic & microscopic evaluation of condition of the cornea
- Assessment of corneal opacity (cloudiness)
- Measurement of corneal thickness (swelling)
- Uptake of sodium fluorescein by the cornea
- Histopathology may be performed if required

Apparatus

Superfusion apparatus

- 11 temperature controlled chambers

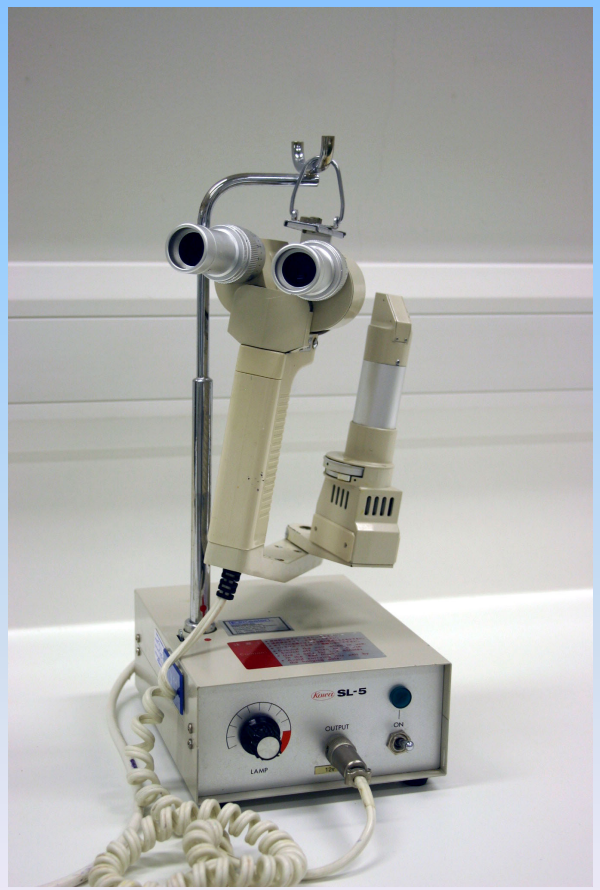
- 4 temperature probes



Material: Perspex or polypropylene

Cost: ~ £ 1200

Apparatus



Portable slit-lamp

Model SL-5

Supplier: Kowa

Cost: ~ £3000

Apparatus

Ultrasonic pachymeter

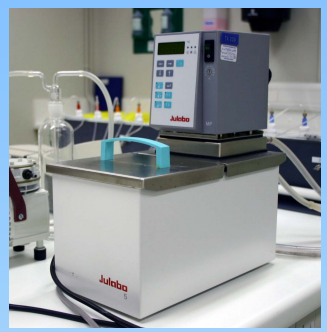
Model DGH-1000

Supplier: DGH Technology, Inc.

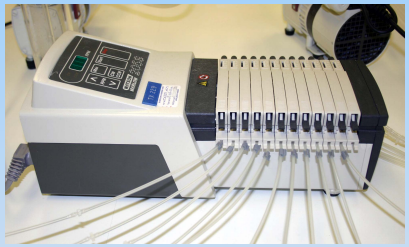
Cost (Model DGH-550): ~ £4000

Apparatus

- Temperature-controlled circulating water bath



- Multi-channel Peristaltic Pump



- Diaphragm pump

- Temperature monitors

Source of Tissues

- Rabbits from local accredited supplier
- Strain: New Zealand White (albino)
- No specified weight or age range. Typically 2.5 – 4.0 kg animals
- May previously have been used for skin irritation tests at Safepharm
- Control eyes from animals that have undergone an eye irritation test

Pre-test Procedures: Apparatus Set-Up

- Temperature of water bath adjusted to achieve a stable temperature of 32 ± 1.5°C
- Flow-rate of peristaltic pump adjusted to 0.15 – 0.4 ml/minute

Pre-test Procedures: In vivo Examination

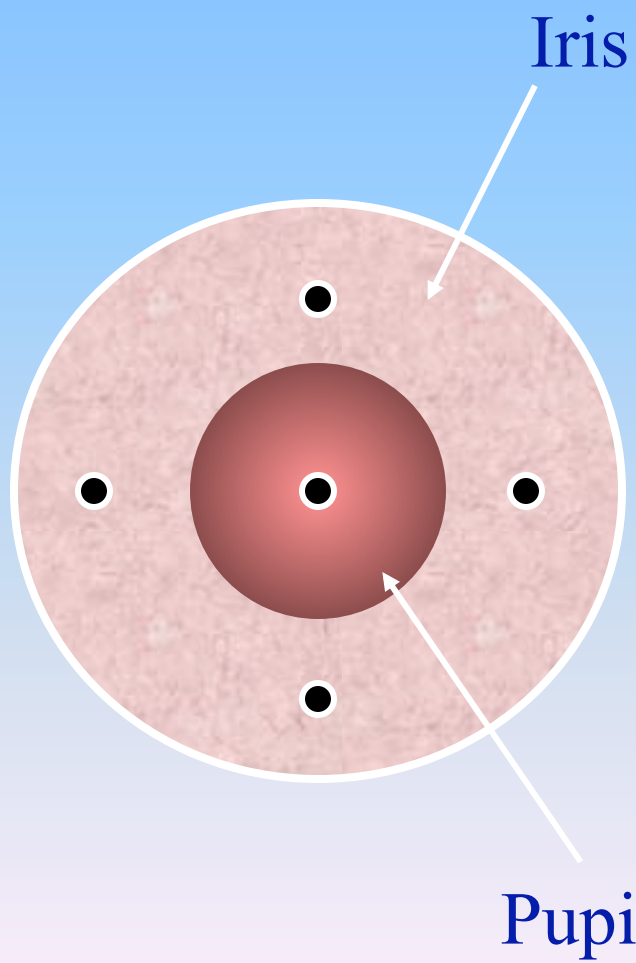
- Application of Sodium Fluorescein (1% w/v) to the rabbit eye + rinse
- Slit-lamp examination of the cornea
- Measurement of corneal thickness (t = -1)

- Animals with corneal defects are rejected

Pre-test Procedures: Corneal Thickness

- Using Ultrasonic pachymeter
- No pain, no distress
- Five positions
- Optical centre
- 3, 6, 9 & 12 o' clock positions
- Mean corneal thickness calculated

(n = 5)

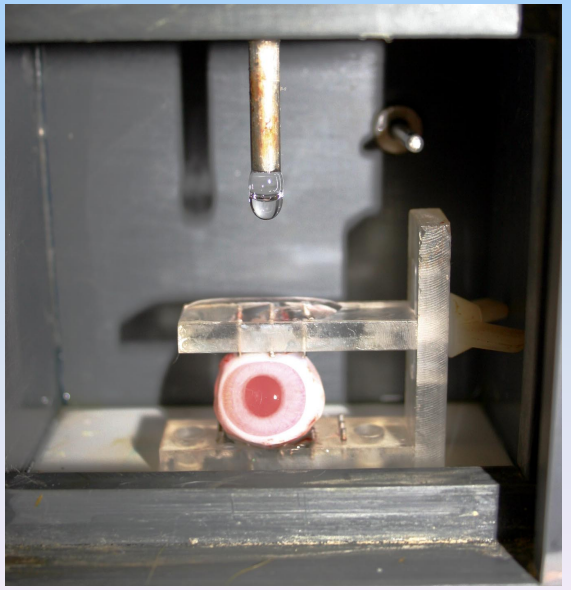


Pre-test Procedures: Enucleation of the Eye

- Animals humanely sacrificed by i.v. overdose of sodium pentobarbitol (marginal ear vein)
- 2-3 drops physiological saline (approximately 32^{o}C) applied to the eye to prevent dessication
- Eye removed by careful dissection

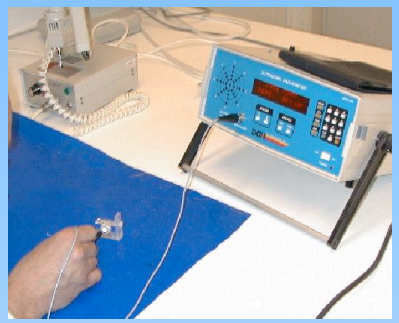
Pre-test Procedures: Mounting of Eye

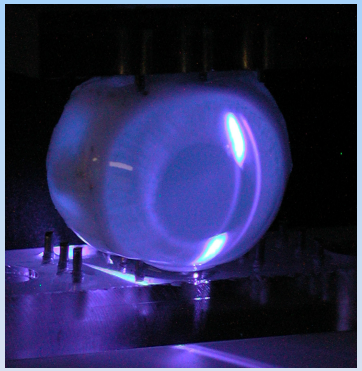
- Eye mounted vertically in perspex clamp (Burton, York & Lawrence, 1980)
- Held in place by adjustable jaws with stainless steel ‘pins’
- Saline drip adjusted if necessary



Pre-test Procedures:Equilibration

- 30 minute equilibration
- Eyes re-examined
  - corneal thickness (t = 0)
  - slit-lamp examination
  - Sodium fluorescein 1%





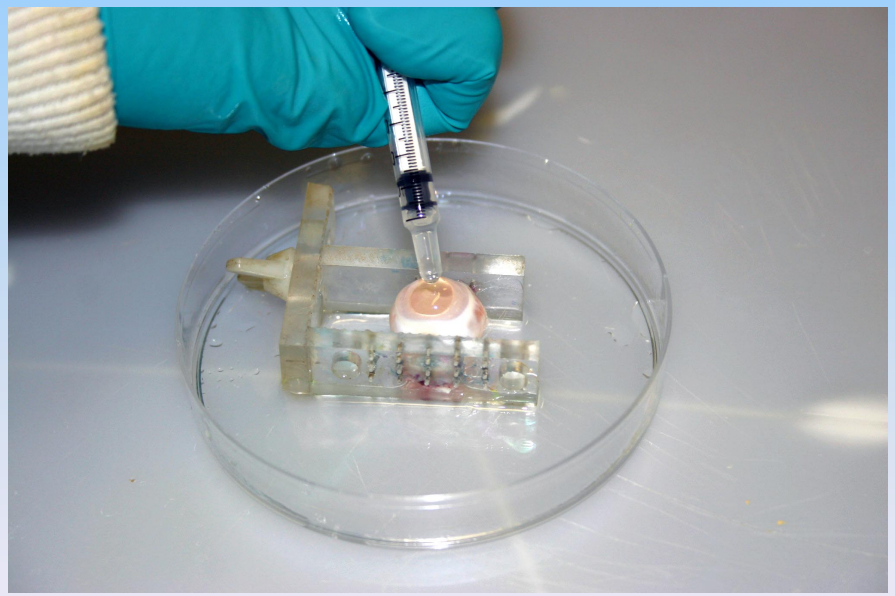
Eyes showing an increase in corneal thickness of ≥10%, from t = -1 are rejected

Method of Test Substance Application

- Eye removed from chamber & placed in petri dish
- Test material applied from a disposable syringe

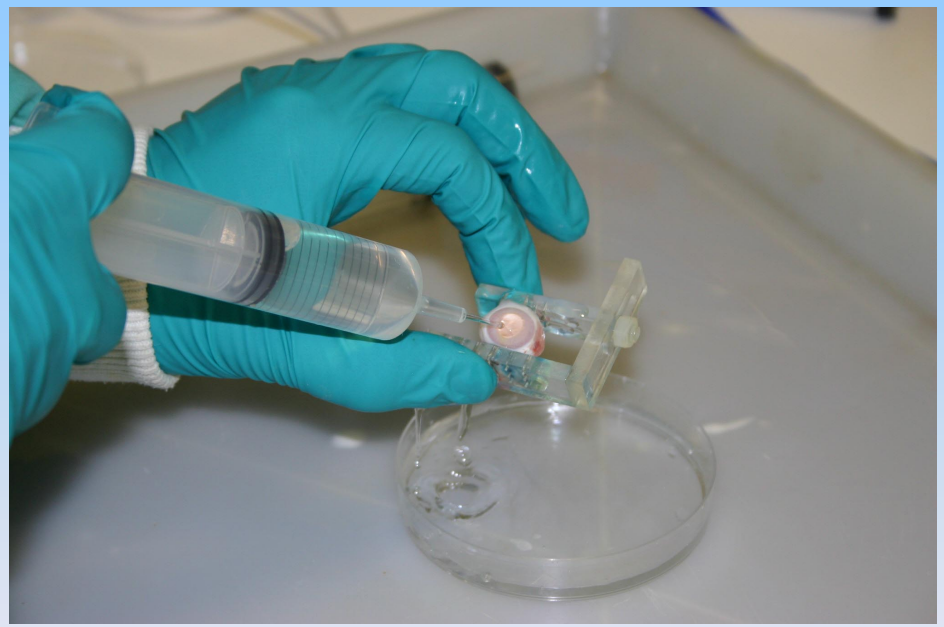
Liquids - 0.1 ml

Solids – volume occupying 0.1 ml
(or maximum of 100 mg)



Method of Test Substance Application

After 10 seconds cornea rinsed with 20 ml of saline (32°C)



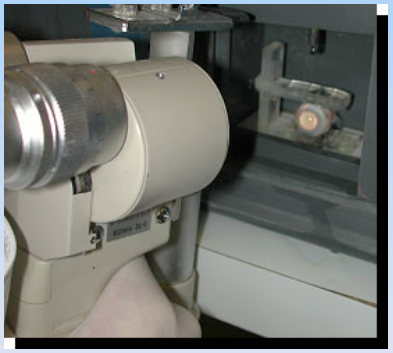
Chemical Range

- Broad range of chemicals and formulations
  - solids
  - liquids
  - pastes
  - various chemical classes
- Same classes of chemicals as the Draize Test
- If material runs off or adheres to the cornea, this is recorded

Observations (1, 2, 3 and 4 hours after treatment)

- Macroscopic (visual) assessment of cornea (diffuse illumination)
- Mottling
- Pitting
- Sloughing of epithelium

- Slit-lamp biomicroscopic examination of the condition of the cornea



- Epithelial & more in depth injuries (stroma & endothelium)

Observations (1, 2, 3 and 4 hours after treatment)

- Corneal opacity (cloudiness)

- Severity & Area
- 0 - 4 scale (Hackett & McDonald, 1991)

Observations (1, 2, 3 and 4 hours after treatment)

Severity of opacity (cloudiness)

Abridged version of the 0-4 scale

0 = Normal

1 = Some loss of transparency

2 = Moderate loss of transparency

3 = Involvement of entire thickness of stroma underlying structures barely visible)

4 = Involvement of entire thickness of stroma (underlying structures cannot be seen)

Observations (1, 2, 3 and 4 hours after treatment)

Area of opacity

0 = Normal cornea with no area of cloudiness

1 = 1 to 25% area of stromal cloudiness

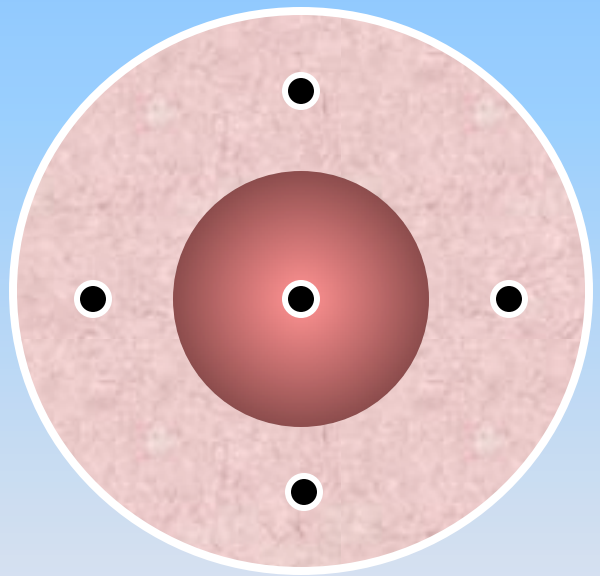
2 = 26 to 50% area of stromal cloudiness

3 = 51 to 75% area of stromal cloudiness

4 = 76 to 100% area of stromal cloudiness

Observations (1, 2, 3 and 4 hours after treatment)

- Measurement of corneal thickness (ultrasonic pachymeter)



- Five positions
- Optical centre
- 3, 6, 9 & 12 o’ clock positions
- Mean corneal thickness calculated for each eye at each observation

Observation (4 hours after treatment)

- Evaluation of sodium fluorescein uptake by the cornea
- Conducted after application of one drop of sodium fluorescein (1% w/v) to the rabbit eye (with rinse)

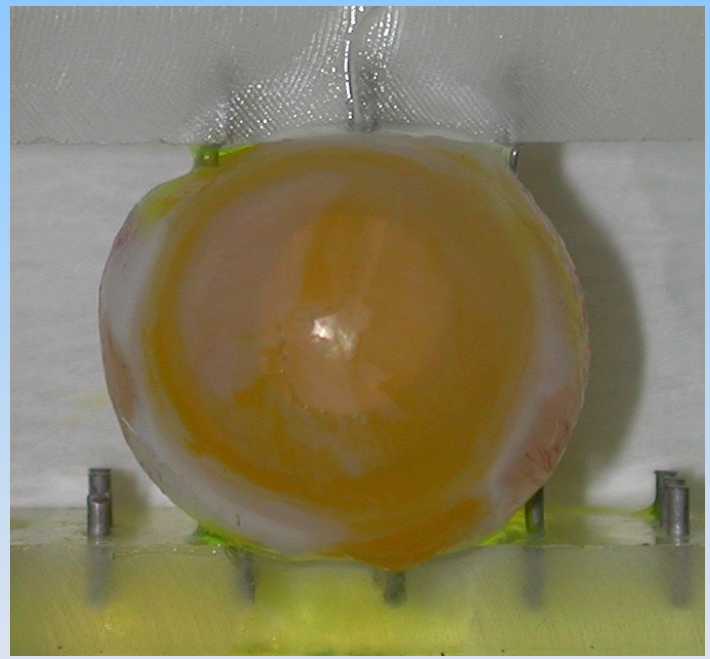
- Degree & Area
- 0 - 4 scale (Hackett & McDonald, 1991)

Observations (4 hours after treatment)

Severity

0 = Absence of fluorescein staining
1 = Slight fluorescein staining
2 = Moderate fluorescein staining
3 = Marked fluorescein staining
4 = Extreme fluorescein staining

Area - as for corneal opacity



IRE: Evaluation of Results

- The percentage increase in corneal swelling is calculated for each eye, at each time point (t = 60, 120, 180 & 240)

$$\frac{(\text{Mean thickness, time t}) - (\text{mean thickness, time 0})}{\text{Mean thickness, time 0}} \times 100$$

- Mean corneal swelling is calculated for the 3 test eyes and 2 control eyes

Prediction Model

An assessment of ocular irritancy potential is made in accordance with the following prediction model

Parameter	Cut-off Value
Maximum Corneal Opacity (cloudiness x area)	≥ 3
Maximum Fluorescein Uptake (Intensity x Area)	≥ 4
Mean Corneal Swelling (60, 120, 180, 240 mins)	≥ 25%
Corneal Epithelium Observations	Any eye with pitting, mottling or sloughing

Prediction Model

- If any of the PM criteria are met, then the test material is regarded as a potential severe eye irritant
- Such materials do not require testing in vivo
- If any of the criteria are met by the control eyes, the test is repeated

Conclusion (1)

- The IRE has been routinely used at Safepharm since 1999 as a screen for severe ocular irritants and corrosives
- It is incorporated into an ethical tiered testing strategy
- It uses normal eyes that would otherwise be discarded
- It has resulted in a reduction in the number of animals exposed to severe irritants

Conclusion (2)

- If used in conjunction with other in vitro tests (e.g. human reconstituted tissue models) it is hoped that complete replacement of the rabbit eye irritation test may be possible
- Efforts to standardise the test protocol and formally validate the model are encouraged

Acknowledgements

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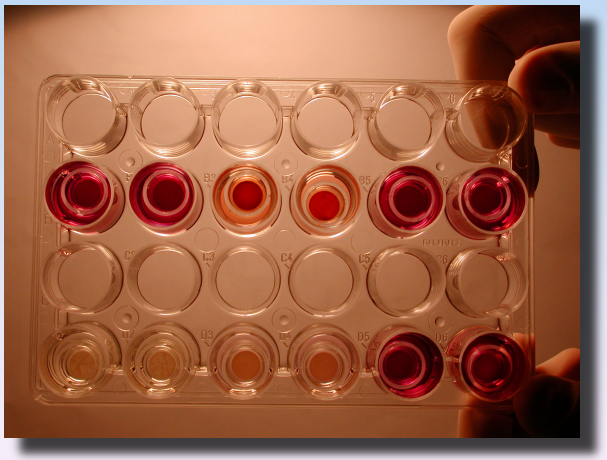
The Isolated Rabbit Eye (IRE) Test Method

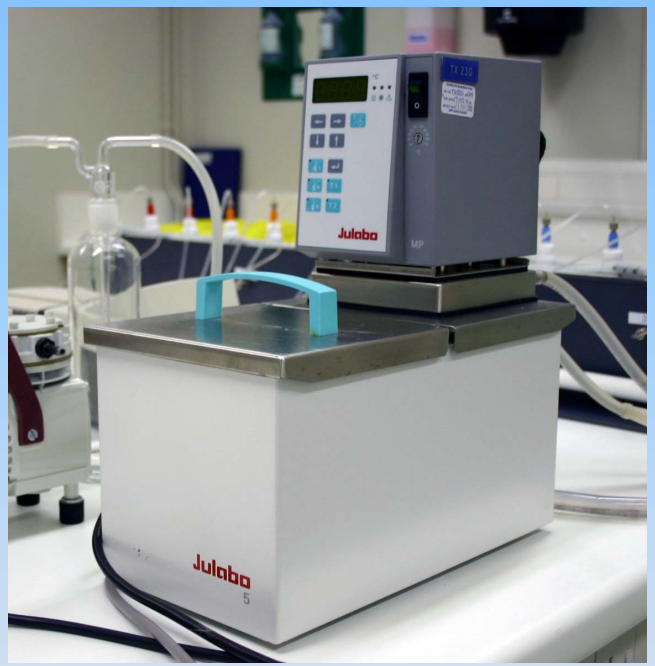
Thank you

Toxicology Testing Services

- Acute Toxicity
- Local Tolerance (skin, eye)
- Skin Sensitisation
- Photosafety
- Sub-Chronic Toxicity
- Reproductive Toxicity
- Genetic Toxicology
- Ecotoxicology
- Alternative Test Methods



Apparatus

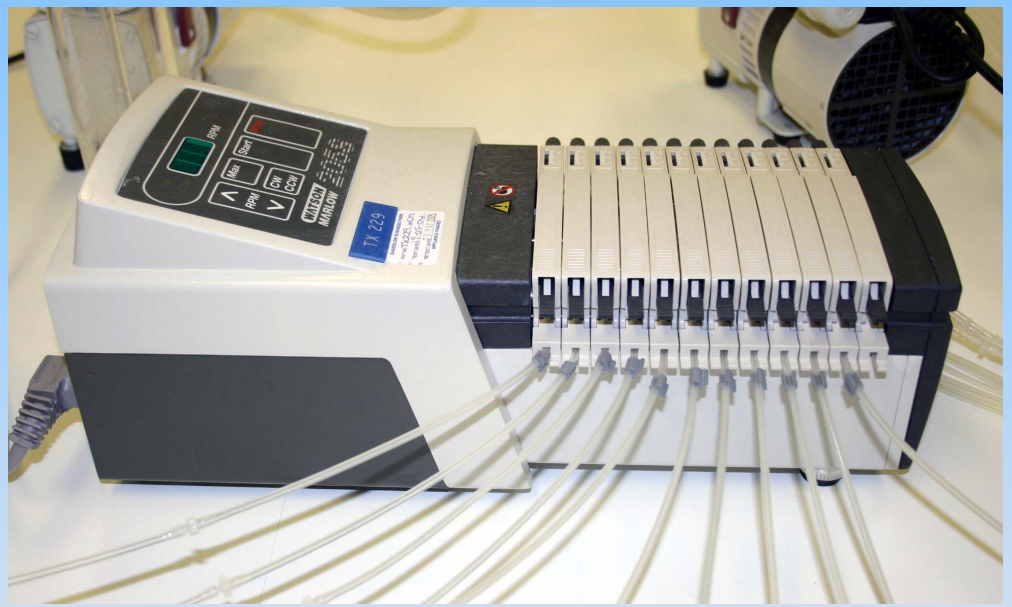


Temperature-controlled circulating water bath

Supplier: Julabo

Cost: ~ £1000

Apparatus



Multi-channel
Peristaltic Pump

Supplier: Watson-Marlow

Cost: ~ £4000

Apparatus

Diaphragm pump

Supplier: KNF Neuberger

Cost: ~ £400

Apparatus

Temperature monitors

Supplier: Widely available

Cost: ~ £ 100 each

Test Area

20 feet x 20 feet

(2 systems)

Total cost of 1 system: £15000

IRE: Observations

1, 2, 3 and 4 hours after treatment

- Macroscopic (visual) assessment of the cornea
- Slit-lamp biomicroscopic examination of the cornea
- Evaluation of corneal cloudiness (severity & area)
- Measurement of corneal thickness (ultrasonic pachymeter)

IRE: Observations

Additional Observation: 4 hours after treatment

- Evaluation of sodium fluorescein uptake by the cornea (severity and area)

- Application of one drop of sodium fluorescein (1% w/v) to the rabbit eye

- Rinsed with physiological saline at 32^{o}C (10 ml)

Observations (4 hours after treatment)

Area of fluorescein staining

0 = Normal

1 = 1 to 25% area of cornea

2 = 26 to 50% area of cornea

3 = 51 to 75% area of cornea

4 = 76 to 100% area of cornea